JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1985, p. 542–545
0095-1137/85/040542-04$02.00/0

Vol. 21, No. 4

Comparison of the API Rapid E Four-Hour System with the API 20E Overnight System for the Identification of Routine Clinical Isolates of the Family *Enterobacteriaceae*

TIMOTHY L. OVERMAN,[1,2]* DANIEL PLUMLEY,[3] SUE B. OVERMAN,[3] AND NORMAN L. GOODMAN[2,3]

Pathology Service, Veterans Administration Medical Center, Lexington, Kentucky 40511,[1] and Department of Pathology[2] and Clinical Microbiology Laboratories,[3] University of Kentucky Medical Center, Lexington, Kentucky 40536

Received 29 November 1984/Accepted 7 January 1985

Four hundred forty-one clinical isolates of the family *Enterobacteriaceae* were identified in parallel by using the API Rapid E 4-h and the API 20E overnight procedures (Analytab Products, Plainview, N.Y.). The results obtained by using the API Rapid E were compared with those obtained by using the API 20E. Discrepancies were resolved by using standard biochemicals. The API 20E identified 98.9% (436 of 441) of the isolates without the use of additional biochemicals and was found to be correct in each case of a discrepancy among the 436 isolates. The API Rapid E gave the same identification as the API 20E for 94.0% (410 of 436) of the isolates, misidentified 3.0% (13 of 436), and gave a correct but low-selectivity answer for the remaining 3.0% (13 of 436). The API Rapid E is a suitable alternative for the rapid identification of the *Enterobacteriaceae*.

There has been a concerted effort to reduce the turnaround time for the identification of microorganisms isolated from clinical specimens by both manual and automated methods. Much of the effort has centered on the family *Enterobacteriaceae* with the development of manual systems such as Micro-ID (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.) (1, 3, 7) and the API 20E same-day identification (Analytab Products, Plainview, N.Y.) (2, 8) and automated systems such as the Autobac-IDX (General Diagnostics) (5, 6) and the AutoMicrobic system with the EBC+ card (Vitek Systems, Inc., Hazelwood, Mo.) (4).

Another manual system for the rapid identification of the *Enterobacteriaceae*, called the API Rapid E (formerly DMS Rapid E) (Analytab Products), has recently been introduced (9, 10, 13). This system consists of a plastic strip with 20 microtubes containing dehydrated biochemical substrates. This report compares the results obtained by using the API Rapid E (RE) 4-h system to identify members of the family *Enterobacteriaceae* isolated from routine clinical specimens with those obtained by using the overnight API 20E procedure.

MATERIALS AND METHODS

Four hundred forty-one clinical isolates of members of the family *Enterobacteriaceae* were identified in parallel by using the API 20E 18-h and the RE 4-h systems. All discrepancies were resolved by the use of standard biochemicals as recommended by the manufacturers of the systems. The organisms included in the study and the number of isolates were as follows: *Citrobacter amalonaticus*, 2; *Citrobacter diversus*, 11; *Citrobacter freundii*, 11; *Edwardsiella tarda*, 1; *Enterobacter aerogenes*, 21; *Enterobacter agglomerans*, 4; *Enterobacter cloacae*, 18; *Enterobacter gergoviae*, 2; *Escherichia coli*, 114; *Hafnia alvei*, 4; *Klebsiella oxytoca*, 19; *Klebsiella ozaenae*, 2; *Klebsiella pneumoniae*, 61; *Klebsiella rhinoscleromatis*, 1; *Morganella morganii*, 6; *Proteus mirabilis*, 61; *Proteus vulgaris*, 4; *Providencia rettgeri*, 9; *Providencia stuartii*, 24; *Salmonella* sp., 14; *Serratia liquefaciens*, 1; *Serratia marcescens*, 41; *Serratia odorifera*, 1; *Serratia plymuthica*, 1; *Shigella* sp., 7; *Yersinia enterocolitica*, 1.

The RE is a plastic strip to which are attached 20 microtubes containing various substrates and carbohydrates (Fig. 1). These substrates are not buffered, and the microtubes are smaller than those of the API 20E (Fig. 1), allowing for more rapid reactions. The inoculum is prepared to the density of a 0.5 McFarland barium sulfate standard. The small total volume required to inoculate the API Rapid E strip makes obtaining this density possible with only one or two well-isolated colonies. The tests are arranged in groups of three, and by using octal numbers, a seven-digit profile number is generated for the 20 tests plus oxidase. The tests contained on the RE strip are described in the legend to Fig. 1. Lysine decarboxylase, ornithine decarboxylase, and urease tests must be overlaid with sterile mineral oil. The only tests requiring the addition of reagents are indole production and acetoin production, which require Kovacs reagent and 40% potassium hydroxide–6.0% alpha-naphthol, respectively. The RE strip oxidase test, the addition of 1.0% *N,N,N,N*-tetramethyl-*para*-phenylenediamine in isoamyl alcohol to either the esculin or phenylalanine deaminase tube with the development of violet color within 5 min, was not used. The Kovacs oxidase method (11) was used to test each isolate before inoculating the RE and the API 20E. The RE comes packaged in its own incubation tray, and sterile plastic pipettes for inoculating the RE strips are also provided. Distilled water was not added to the RE incubation trays. All of the required reagents are available from the manufacturer.

The API 20E, also shown in Fig. 1, has been extensively described (1, 3, 7, 14, 15, 16) and will not be described here except to note that it has the following tests in common with the RE: *o*-nitrophenyl-β-D-galactopyranoside; lysine decarboxylase; ornithine decarboxylase; citrate utilization; urease; production of indole and acetoin; fermentation of glucose, sucrose, melibiose, and arabinose.

* Corresponding author.

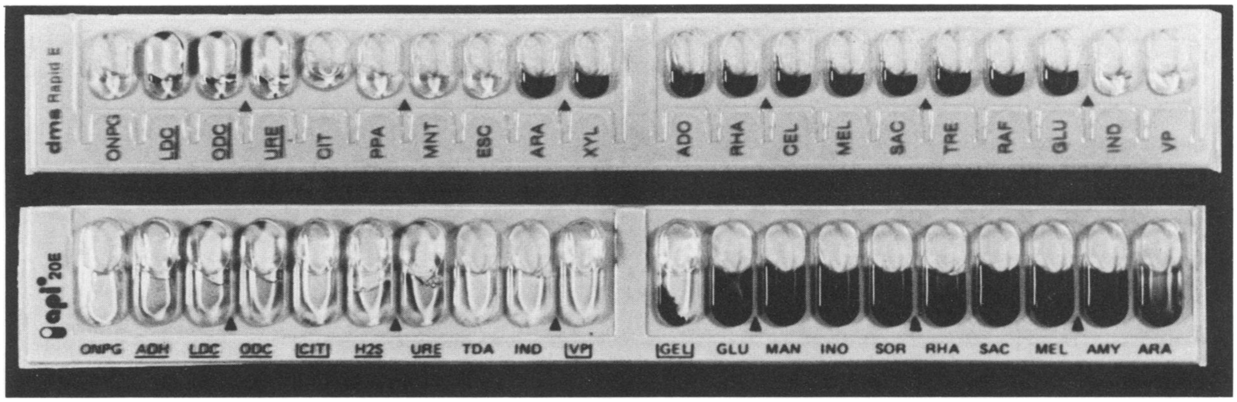

FIG. 1. API RE (top) and API 20E (bottom), inoculated, but not incubated. RE tests: *o*-nitrophenyl-β-D-galactopyranoside (ONPG); lysine decarboxylase (LDC); ornithine decarboxylase (ODC); urease (URE); citrate utilization (CIT); phenylalanine deaminase (PPA); malonate utilization (MNT); esculin hydrolysis (ESC); fermentation of arabinose (ARA), xylose (XYL), adonitol (ADO), rhamnose (RHA), cellobiose (CEL), melibiose (MEL), sucrose (SAC), trehalose (TRE), raffinose (RAF), and glucose (GLU); production of indole (IND) and acetoin (VP). Note the differences in the size of the microtubes, the tests used, and the order of tests.

Bacteria from one isolated colony growing on primary isolation medium (5% sheep blood agar, chocolate agar, or MacConkey agar) were suspended in 5 ml of 0.85% sterile saline to serve as the inoculum for the API 20E. A bacterial suspension approximating the turbidity of a 0.5 McFarland barium sulfate standard was prepared in 1.25 ml of 0.85% sterile saline to serve as the inoculum for the RE. Only one to two isolated colonies growing on primary isolation medium were required to obtain the proper inoculum density for the RE (9, 10, 13).

The API 20Es and the REs were inoculated and incubated at 35°C in accordance with the instructions of the manufacturers. After 4 h of incubation, reagents were added to the REs and a seven-digit profile number was generated in accordance with the instructions of the manufacturer for test interpretation. After 18 to 24 h of incubation, reagents were added to the API 20Es, and a seven-digit profile number was generated in accordance with the instructions of the manufacturer for test interpretation.

The RE and API 20E identifications were made by finding the profile numbers in the Rapid E *Enterobacteriaceae* identification codebook and the API 20E analytical profile index for *Enterobacteriaceae* and other gram-negative bacteria, respectively. If the profile number was not found, then the appropriate computer identification service was called.

RESULTS

The API 20E identified 98.9% (436 of 441) of the isolates without the use of additional biochemicals. Four of the five isolates not identified by the API 20E were *Citrobacter diversus*, all having the same profile number. The fifth isolate was a *Providencia rettgeri*. The API 20E identification was correct in each case of a discrepant identification between the two systems for the 436 isolates identified by the API 20E without the use of additional biochemicals.

The RE gave the same identification as the API 20E for 410 of those 436 isolates for an overall identification rate of 94.0%. There were 13 isolates of the 436 for which the RE identification code listed the correct identification as one of several possible choices, but the quality of the identification was only acceptable or of low discrimination (<90.0%). The low-selectivity identification rate was 3.0%. The remaining 13 of the 436 isolates were incorrectly identified for an error rate of 3.0%. The distribution of correct, incorrect, and low-selectivity identifications is shown in Table 1.

DISCUSSION

The purpose of this study was to compare the results obtained with the RE in a routine clinical setting with those of the API 20E. The API 20E has been extensively evaluated (1, 3, 7, 14–16) and has gained widespread acceptance and use. In fact the API 20E has been used as the comparison standard in other evaluations (6, 12). The decision was made not to do an evaluation with large numbers of rarely isolated organisms, as the usefulness of any identification system is its ability to identify the most commonly isolated or routine organisms.

The API 20E identified, acceptable (>90.0%) to excellent categories, 98.9% (436 of 441) of the clinical isolates without the need of additional biochemicals. Since the API 20E was the standard system in this comparison, it was decided not to expect the RE to identify isolates which were not identified by the API 20E. Therefore, the comparison is based on the 436 isolates which were identified by the API 20E.

The RE system had a 94.0% identification agreement with the overnight API 20E and provided correct but low-selectivity (<90.0%; RE acceptable or low-discrimination categories) answers for 3.0% of the isolates. The misidentification rate was also 3.0%. Of the 13 misidentifications, 11 had identification qualities of >90.0%. Four of these 11 were correct to the genus level; 7 were not. The remaining two misidentifications were of low selectivity. One of these two listed the correct genus as a possible choice; the other did not.

The identification rate is comparable to those reported for the RE system in two recently published studies. Izard et al. (9) studied 567 clinical isolates and reported an identification rate of 95.9%, a misidentification rate of 2.5%, and a failure-to-identify rate of 1.4%. Murray et al. (13) studied 492 clinical isolates and reported an identification rate of 94.1%, a misidentification rate of 3.9% (19 of 492), and a failure-to identify or low-selectivity identification rate of 2.0% (10 of 492). The majority of the RE identification problems occurred within the tribe *Klebsielleae*. This problem was also reported by Izard et al. (9) and Murray et al. (13).

A third recently published study (10) reported a higher identification rate of 97.2% for 387 clinical isolates. The

TABLE 1. Distribution of API RE identifications

Organism as identified by API 20E[a]	No. of cultures identified as:																											
	C. amalonaticus	*C. diversus*	*C. freundii*	*E. tarda*	*E. aerogenes*	*E. agglomerans*	*E. cloacae*	*E. gergoviae*	*E. coli*	*H. alvei*	*K. oxytoca*	*K. ozaenae*	*K. pneumoniae*	*K. rhinoscleromatis*	*M. morganii*	*P. mirabilis*	*P. vulgaris*	*P. rettgeri*	*P. stuartii*	*Salmonella* sp.	*S. liquefaciens*	*S. marcescens*	*S. odorifera*	*S. plymuthica*	*Shigella* sp.	*Y. enterocolitica*	Low selectivity, correct	Low selectivity, incorrect
Citrobacter amalonaticus	2																											
C. diversus		7																										
C. freundii			9				1																				1	
Edwardsiella tarda				1																								
Enterobacter aerogenes					21																							
E. agglomerans						1	1		1																			1
E. cloacae					1		15																				2	
E. gergoviae								2																				
Escherichia coli					1				112																		1	
Hafnia alvei										4																		
Klebsiella oxytoca											18		1															
K. ozaenae												2																
K. pneumoniae							1		1				56														3	
K. rhinoscleromatis																												1
Morganella morganii															5												1	
Proteus mirabilis																61												
P. vulgaris																	3										1	
Providencia rettgeri															1			4									3	
P. stuartii																			24									
Salmonella sp.																				14								
Serratia liquefaciens																						1						
S. marcescens																						41						
S. odorifera											1																	
S. plymuthica																								1				
Shigella sp.																									7			
Yersinia enterocolitica																											1	

[a] Organisms identified by API 20E were tested with standard biochemicals to resolve discrepant results between API 20E and API Rapid E. API 20E was correct in all such cases. Five isolates not identified by API 20E are not included.

remaining 2.8% of misidentifications can be calculated into rates of 0.3% (1 of 387) for misidentification and 2.5% (10 of 387) for no identification or low selectivity. These better rates of identification and misidentification may be a factor of the organism composition of the 387 isolates. There were only five species of the tribe *Klebsielleae* represented, constituting 26.4% (102 of 387) of the isolates. The study of Izard et al. (9) contained 12 species of the tribe *Klebsielleae*, which constituted 42.9% (243 of 567) of the isolates. Nine species of the tribe *Klebsielleae* constituted 51.0% (251 of 492) of the isolates in the study of Murray et al. (13). In our study, there were 13 species of the tribe *Klebsielleae* represented which constituted 40.4% (176 of 436) of the isolates. Also, in the study of Keville and Doern (10), *Escherichia coli* strains constituted 53.0% of the 387 isolates, whereas in our study, only 26.1% of the isolates were *E. coli*. The two other previous studies had 5.3% (9) and 26.0% (13) *E. coli* isolates. The identification rate for *E. coli* has varied from a low of 97.6% (13) to 100% (9). The identification rate for *E. coli* reported by Keville and Doern (10) was 99.5%; our *E. coli* identification rate was 98.2%.

As shown in Table 1, the RE strip is an acceptable alternative for the identification of most commonly occurring members of the family *Enterobacteriaceae*. Those isolates which give an RE code of low selectivity or are unidentified can be identified by using an overnight method such as the API 20E. The number of isolates requiring such additional identification procedures should be a distinct minority of the commonly occurring *Enterobacteriaceae* isolates.

ACKNOWLEDGMENTS

We thank Phyllis Campbell, Janet Kessler, and Brian Willcut for their technical assistance.

LITERATURE CITED

1. **Aldridge, K. E., B. B. Gardner, S. J. Clark, and J. M. Matsen.** 1978. Comparison of Micro-ID, API 20E, and conventional media systems in identification of *Enterobacteriaceae*. J. Clin. Microbiol. **7**:507–513.
2. **Appelbaum, P. C., R. R. Arthur, M. E. Parker, G. L. Shugar, L. C. Von Kuster, and P. Charache.** 1982. Comparison of two methods for same-day identification of *Enterobacteriaceae*. Am. J. Clin. Pathol. **78**:351–355.
3. **Barry, A. L., and R. E. Badal.** 1979. Rapid identification of *Enterobacteriaceae* with the Micro-ID system versus API 20E and conventional media. J. Clin. Microbiol. **10**:293–298.
4. **Barry, A. L., and R. E. Badal.** 1982. Identification of *Enterobacteriaceae* by the AutoMicrobic system: *Enterobacteriaceae* Biochemical Cards versus *Enterobacteriaceae*-plus Biochemical Cards. J. Clin. Microbiol. **15**:575–581.

5. **Barry, A. L., T. L. Gavan, P. B. Smith, J. M. Matsen, J. A. Morello, and B. H. Sielaff.** 1982. Accuracy and precision of the Autobac system for rapid identification of gram-negative bacilli: a collaborative evaluation. J. Clin. Microbiol. **15:**1111–1119.
6. **Costigan, W. J., and G. E. Hollick.** 1984. Use of the Autobac IDX system for rapid identification of *Enterobacteriaceae* and nonfermentative gram-negative bacilli. J. Clin. Microbiol. **19:**301–302.
7. **Edberg, S. C., B. Atkinson, C. Chambers, M. H. Moore, L. Palumbo, C. F. Zorzon, and J. M. Singer.** 1979. Clinical evaluation of the Micro-ID, API 20E, and conventional media systems for the identification of *Enterobacteriaceae*. J. Clin. Microbiol. **10:**161–167.
8. **Gooch, W. M., III, and G. A. Hill.** 1982. Comparison of Micro-ID and API 20E in rapid identification of *Enterobacteriaceae*. J. Clin. Microbiol. **15:**885–890.
9. **Izard, D., M. O. Husson, P. Vincent, H. Leclerc, D. Monget, and J. M. Boeufgras.** 1984. Evaluation of the four-hour rapid 20E system for identification of members of the family *Enterobacteriaceae*. J. Clin. Microbiol. **20:**51–54.
10. **Keville, M. W., and G. V. Doern.** 1984. Evaluation of the DMS Rapid E system for identification of clinical isolates of the family *Enterobacteriaceae*. J. Clin. Microbiol. **20:**1010–1011.
11. **Kovacs, N.** 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) **178:**703.
12. **Malloy, P. J., M. J. Ducate, and P. C. Schreckenberger.** 1983. Comparison of four rapid methods for identification of *Enterobacteriaceae* from blood cultures. J. Clin. Microbiol. **17:**493–499.
13. **Murray, P. R., A. Gauthier, and A. Niles.** 1984. Evaluation of the Quantum II and Rapid E identification systems. J. Clin. Microbiol. **20:**509–514.
14. **Nord, C. E., A. A. Lindberg, and A. Dahlback.** 1974. Evaluation of five test-kits—API, AuxoTab, Enterotube, PathoTec, and R/B—for identification of *Enterobacteriaceae*. Med. Microbiol. Immunol. **159:**211–220.
15. **Smith, P. B., K. M. Tomfohrde, D. L. Rhoden, and A. Balows.** 1972. API system: a multitube micromethod for identification of *Enterobacteriaceae*. Appl. Microbiol. **24:**449–452.
16. **Washington, J. A., II, P. K. W. Yu, and W. J. Martin.** 1971. Evaluation of accuracy of multitest micromethod system for identification of *Enterobacteriaceae*. Appl. Microbiol. **22:** 267–269.